BODY WEIGHT, SURVIVAL TIME, COLORATION, AND WATER CONTENT OF SKELETAL MUSCLES OF ADRENALECTOMIZED FROGS[1]

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From studies of slightly more than 1300 operated frogs, of which between 65% and 70% were adrenalectomized and the remainder were renal damaged ("sham"-operated controls), various observations relative to changes in body weight, survival time, integumental coloration, and water content of skeletal muscles are available to extend, as well as to correlate, the prevailing literature relative to the effect of adrenalectomy in frogs.

Male *Rana pipiens* weighing between 30 and 35 g. may be arbitrarily divided into four groups: adrenalectomized frogs whose postoperative body weights were either (a) *controlled* to be within ± 1.5 g. of their respective mean preoperative values, or (b) *uncontrolled;* and the control frogs—(c) *renal damaged*, and (d) *unoperated*. During an interval from 10 days prior to adrenalectomy until the time of death, individual daily records were kept with regard to various pertinent data. Because of the subjective nature of interpreting integumental color changes, the same two persons[2] always made the observations.

All frogs were unfed. The appearance of ample testicular fat at autopsy was considered as evidence of at least a "basal" state of nutrition; otherwise the frogs were excluded from the results.

Data derived from the four groups of frogs are summarized in Fig. 1. The various mean values for the respective data for each group (25 frogs/group) have been statistically compared. Significance is considered to exist when $P<0.05$.

Body weights. The percentage differences between the postoperative values of the body weights for each group of frogs designated in the legend under Fig. 1 and their respective preoperative values are plotted as functions of the postoperative time in days. The uncontrolled adrenalectomized frogs, unlike the three remaining groups, show a progressive increase in mean body weight so that by the seventh and twelfth days following adrenalectomy there is a significant increase of 28 and 33% respectively. Given sufficient time in an aqueous environment, the adrenalectomized frog undergoes a pronounced swelling [cf. Maes (1), p. 145]. This swelling is not only reflected in "un oedeme généralisé" but also in a cellular imbibition of fluid as shown macroscopically by the swollen, milky-white appearance of the myofibers of skeletal muscles. The progressive gain in body (water) weight becomes significant on the second day following adrenalectomy. This is interesting in light of the increased rate at which certain vital dyes and tagged ions penetrate adrenalectomized frog skin (2). That this increase is due to lack of the adrenocortical hormone and not to the localized kidney damage which tends to accompany cauterization is supported by studies on injury to this organ. Ordinarily the adrenal tissue can be "wiped" from the kidney by the convex side of a curved cautery needle. When "sham" operated frogs are damaged by longitudinally cauterizing both kidneys to a depth and width greater than necessary for removal of the adrenal, one finds no difference between the two groups of controls as regards

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the variables under discussion. Indeed, this was true even when both kidneys were deliberately split except for a segment a few mm. long at both poles, which served to bind the lateral portion of the kidney to its medial mass. That this drastic procedure is apparently not deleterious to the frog is inferred from studies in which the ureters were bilaterally ligated (3).

Survival time. Sixty-seven per cent of the uncontrolled adrenalectomized frogs of the fall and winter stock, when kept at room temperature (17°–22° C.), died between the seventh and twelfth days postoperatively. Studies on summer and early fall frogs support Maes' (1) findings that survival is reciprocally related to environmental temperature. Spring frogs do not survive adrenalectomy as well as frogs in other seasons; possibly due to a form of stress arising during the breeding season. Indeed, "the adrenalectomized animal is abnormally sensitive to every type of stress" (4).

To test whether the abnormal increase in water load of uncontrolled adrenalectomized frogs served to alter the death point, adrenalectomized frogs were kept over sphagnum moss wetted to a degree determined from the daily observation of individual body weights. In this way, body weights were fairly well controlled (Fig. 1; cf. Adolph (5), p. 119). Cognizant of the susceptibility of adrenalectomized animals to many forms of stress, especially an increase (6, 7) or a decrease (8, 9) in water-load, it was surprising that both groups of frogs showed essentially the same duration of survival following adrenalectomy. As a result of the latter studies (9), the conclusion is reached that the rate of water shift, or of change in osmotic pressure, is the more effective factor in this type of stress.

Coloration. The horizontal bar above the curves (Fig. 1) shows the mean postoperative time in days when an apparent darkening of the dorsal integument was observed in uncontrolled adrenalectomized frogs. The mean time for the appearance of integumental darkening is the end of the second day. Thereupon a succession of characteristic integumental color changes occurred in the following sequence: green, gray-green, gray-brown, gray, and black. Evidently, Maes' (1) statement, "ce changement de pigmentation n' a pas été constant," needs revision, for adrenalectomized frogs given free access to water, and sufficient time to accrue a water load, showed the characteristic sequence of color change. Controlled adrenalectomized frogs darken, though not beyond the gray-brown (usually a gray-green) stage for comparable illumination and background. Darkening of the ventral integument occurs simultaneously, but, of course, to a lesser degree than found on the dorsum. The cause of the color change is unknown, though it is related to water load, since controlled adrenalectomized frogs do not show blackening. Hence it is suggested that this characteristic color change is related to excessive water load, which, in some as yet unknown way, affects the production of melanophore-dispersing hormone (intermedin) by the pituitary.

Skeletal muscle. Since it has been shown that there is a progressive increase in body weight of adrenalectomized frogs given free access to an aqueous environment, and that part of this water is extracellular, it is of interest to compare the water content of skeletal muscles obtained from the four groups of frogs. Pairs of sartorius muscles were rapidly excised, blotted to remove excess water, and weighed with a torsion balance by the same person. The muscles were dried for 18–22 hours at 105° C., and dry weights were determined. The mean percentage of water content for sartorii of each group follows: uncontrolled (84.6 ± 0.4) and controlled (82.8 ± 0.4) adrenalectomized frogs, and their controls . . . renal damaged (82.8 ± 0.4) and unoperated (82.0 ± 0.8) frogs. A statistical comparison of the mean values of the first group of frogs with each of the remaining groups gives a decided significance ($P<0.01$).

Although sartorii from the uncontrolled adrenalectomized frogs have gained 1.8% in water content ($P<0.01$), the intact frog from which these muscles were excised showed a net gain of 23.1% in water content ($P<0.01$). Thus, while the skeletal muscles, intra- and extra-cellularly, tend to absorb water after adrenal-

ectomy, the muscles are not the sole reservoir. This is amply confirmed by the following observations. When a water-loaded, uncontrolled adrenalectomized frog is pithed, upon withdrawal of the needle, there issues from the site of puncture, undoubtedly because of the pressure exerted manually in holding the frog, a stream of fluid for a distance of *ca.* 3–5 mm.

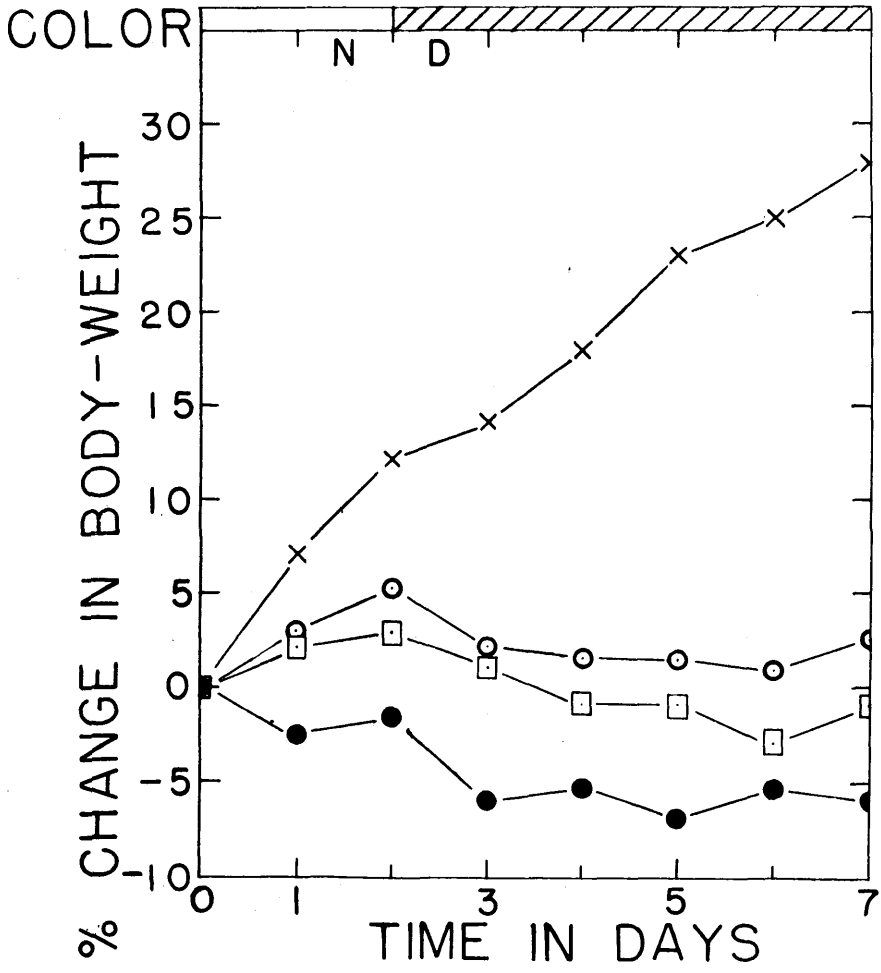

FIG. 1. Percentage changes in body weight of 4 groups of frogs plotted as functions of time. The groups are designated as follows: the adrenalectomized frogs—controlled (•) and uncontrolled (X); the control frogs—renal damaged ("sham" operated) (O) and unoperated (□). The terminations of the curves denote the mean death point for the respective groups; the shaded area of the horizontal bar over the curves signifies the mean time (days) for the initial appearance and duration of integumental darkening (D) in uncontrolled adrenalectomized frogs as compared to "normal" (N) coloration.

These results on frogs are in line with those on mammals (6), and show that the lack of adrenocortical hormone results in an imbalance in the distribution of body fluids, with an accompanying increase in the water content of the liver and the eviscerated carcass of rats (9), of skeletal muscles of rats (11) and frogs (12), and of enucleated red cells of dogs (13), cats (14), and rats (15).

SUMMARY

From a study of slightly more than 1300 operated male grass frogs, of which between 65% and 70% were adrenalectomized and the remainder were renal damaged ("sham" operated) the following conclusions may be drawn.

1. Adrenalectomized frogs whose body weights are permitted to change *ad libitum* show a progressive increase in body weights when placed in a copious aqueous environment, so that on the 7th and 12th days postoperatively there is a mean increase of 28% and 33%, respectively.

2. The mean rate of increase in water load and the total water load absorbed following adrenalectomy in the frog is a function, within limits, of the available water free to enter the integument from the external environment.

3. At room temperature (17°–22° C.), 67% of the adrenalectomized frogs die between the 7th and 12th days postoperatively.

4. The duration of survival following adrenalectomy in the frog is independent of the total increase in water load. Adrenalectomized frogs whose body weights were controlled to within ±1.5 g. of their respective, preoperative, mean values for body weights showed the same survival time, statistically, as did adrenalectomized frogs whose body weights were uncontrolled.

5. As originally observed by Maes (1) these frogs showed integumental blackening after adrenalectomy but contrary to Maes' observation, blackening is not a sporadic occurrence. It is a function of the total increase in water load following adrenalectomy, for adrenalectomized frogs whose body water loads were "controlled" failed to show integumental blackening.

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OHIO'S STATUS AS A GAME AND FUR PRODUCING STATE—CORRECTION

In the March, 1950, issue of the OHIO JOURNAL OF SCIENCE, I discovered the following errata in my article entitled "Ohio as a Game and Fur Producing State:" On page 91, the last sentence under the heading of *Mourning dove* should appear as the second sentence under the heading *Bob-white quail.* Also, under *Mourning dove,* the third sentence should read, "Like the bobwhite, however, the dove, as a game bird in Ohio, was given protection from hunting 1913 to 1917 (rather than 1913 to 1947) and, in the latter year, was designated a song bird (Dambach, 1948)."

My name, although spelled correctly under the title of the article, page 88, had the middle initial J. in it rather than the correct initial L. at the top of pages 90, 92 and 94.—*Daniel L. Leedy.*